1968

Chemical, Physical and Biological Properties of Four Strains of Sugarcanemosaic Virus.

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CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES OF FOUR STRAINS OF SUGARCANE MOSAIC VIRUS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Botany and Plant Pathology

by
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B.S., Southeastern Louisiana College, 1963
M.S., Louisiana State University, 1966
May, 1968

ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to Dr. Thomas P. Pirone, under whose guidance this research has been conducted. The writer also wishes to express his sincere appreciation to Dr. I. L. Forbes for his constructive criticism in the preparation of this manuscript and for serving as committee chairman in the absence of Dr. Pirone. Appreciation is extended to Dr. M. T. Henderson, Dr. Gordon Holcomb, and Dr. Louis Anzalone, members of the examining committee.

Appreciation is also extended to Dr. S. J. P. Chilton, Head, Department of Botany and Plant Pathology for making funds and facilities available.

The writer wishes to thank John Ivey for the photographic work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Several strains of sugarcane mosaic virus (SCMV) have been described using differential host varieties. In studies reported here, an attempt was made to separate the four common strains of SCMV (A, B, D, and H) using chemical, physical and biological properties.

Studies of the physical properties of the four strains showed that thermal inactivation points (TIP) are of no value in strain differentation. All strains were still active at 55°C, but not at 57°C. Dilution end point (DEP) studies revealed a difference in certain of the virus strains in their tolerance to dilution. Strain A and H were still infectious at 10^{-4}, strain D at 10^{-3}, and strain B at 10^{-2}.

A severe leaf necrosis developed on plants infected with certain of the virus strains. Necrosis occurred on sorghum plants infected with strains A, D and H, but not on plants infected with strain B. There was a correlation between the presence of the leaf necrosis and virus concentration.

Three methods of purification were compared to determine the one best suited for use with SCMV. A modification of the method of Delgado-Sanchez and Grogan for potato virus Y yielded the highest amount of infectious virus. Virus purified by this method had less host contaminating material than with other methods tested.

Serological studies were made of the four SCMV strains as well as the Johnson grass mosaic. Results obtained from microprecipitin

tests showed that none of the virus strains could be differentiated using this technique. In these tests, all strains appeared to be closely related. Agar diffusion tests showed that strains A, D and H are closely related. Antigen of strain B did not react with antisera to any of the virus strains including its own. Microprecipitin and agar diffusion tests showed that the Johnson grass mosaic in Louisiana is serologically related to SCMV, but not as closely as are the strains to each other. Spurring in agar diffusion tests indicate that it is a distinct strain of SCMV.

Studies showed that SCMV can be transmitted from infected plants to noninoculated plants through the soil. Transmission occurred in the absence of root contact. The involvement of a biological vector in soil transmission remains to be demonstrated.

INTRODUCTION

Sugarcane mosaic virus (SCMV), affects sugarcane (Saccharum officinarum L.) and certain other members of the Gramineae. The disease caused by this virus has been known for over 70 years, although, it was not until 1920 that its viral nature was shown by Brandes (17).

Electron microscopy of leaf dip and partially purified preparations have shown SCMV to be a flexuous rod with a length of about 750 mu (32, 38, 57). This places SCMV in the potato virus y (PVY) group of plant viruses in Brandes' (18) system of classification. The viruses in this group are all serologically related and have "normal lengths" of 730-790 mu.

SCMV, like most viruses, is probably composed of a number of strains. Several strains of SCMV have been described through the use of differential host varieties. Summers (72, 73) and Summers, Brandes and Rands (74) described ten strains and substrains based on the symptoms produced on the sugarcane varieties Co. 281, C.P. 29-291 and C.P. 31-294. Abbott and Tippett (5) used C.P. 31-294 to differentiate strains A, B, D, E, and F, and C.P. 31-588 to differentiate strains A and H.

The use of differential host varieties has been used effectively to differentiate strains of certain plant viruses. However, several workers are of the opinion that differentiation of strains on the basis of macroscopic symptoms is of limited usefulness (5, 13).

Bawden (12) states that "many virus workers are reluctant to appreciate that variability, especially in symptomatology and host range, is normal rather than exceptional." Factors such as environmental conditions affect the symptoms produced and unless the environment is defined, conditions may be described which may never again be precisely reproduced. According to Bennett (13), serology is more accurate than differential host varieties in establishing strain relationships.

Serology has been used by workers in differentiating strains of many plant viruses (11, 31, 34, 65, 86). Bawden (12) is of the opinion that serology is the most useful and accurate means of allocating strains to a given collective species. The use of serology was limited by early failures to demonstrate serological reactions with some viruses. Recently, better techniques for preparing antigens, along with better serological tests have made serology more applicable for use with plant viruses.

Desai (27), Perez and Adsuar (56) were the first to show the antigenic nature of SCMV. Perez and Adsuar (56) and Abbott and Tippett (5) suggested that serology might be used in demonstrating possible strain relationships. Until recently, however, serological studies with SCMV have not been feasible due to the absence of a suitable method of virus purification. Recently, Pirone and Anzalone (57) and Shepherd (66) have purified SCMV and have obtained antisera of sufficiently high titer for use in serological studies.

The physical properties of a virus are often helpful in differentiation of strains. A number of workers have investigated the

physical properties of SCMV (1, 5, 6, 22, 23) and have reported a wide range of values, both for the thermal inactivation point (TIP) and the dilution end point (DEP). Abbott and Tippett (5) in a recent study of the physical properties of SCMV strains, reported differences in the TIP and DEP values for different strains, but concluded that the use of physical properties in strain differentiation was limited.

This paper describes a study of the chemical, physical and biological properties of four common strains of SCMV. Also, the Johnson grass strain (68, 83) of SCMV, recently reported in Louisiana (58) was included to determine its relationship to the SCMV.

During the course of this study, certain events indicated that SCMV was being transmitted from plant to plant through the soil. Tests were made to determine if SCMV was being transmitted in a soil-borne manner.

LITERATURE REVIEW

According to Matz (49), mosaic was first reported as a sugarcane abnormality by Von Musschenbroek in Java in 1892 where it was known as "gelestrepenzike" or yellow stripe disease. Although the disease had been known for a long time, it was not until 1920 that its infectious nature was demonstrated. Brandes (17) was able to transmit the disease both mechanically and with insects. Brandes showed for the first time, under controlled conditions, that the cell sap of diseased plants is infectious when introduced into the young tissues of healthy plants.

The symptoms of mosaic, which vary in intensity on different varieties, are usually an irregular mottling, with islands of darker green on a background of paler green or yellowish chlorotic areas (28). Edgerton (28) states that symptoms are influenced by the cane variety, the condition of growth, the temperature, and the strain of the virus involved.

The host range of SCMV is limited to members of the grass family. Summers, Brandes, and Rands (74) list 10 cultivated and 34 wild grass hosts of SCMV. Four of the records on cultivated hosts represented observations and six were experimental transmissions, while 19 of the records on wild hosts were observations and 16 were experimental transmissions. The cultivated grasses reported as susceptible to SCMV by experimental transmission are: Andropogon sp., Miscanthus

sinensis Anderss, Pennisetum glaucum (L.) R. Br., Sorghum vulgare var. sudanense (Piper) Hitch., and Zea mays L. The wild grasses reported susceptible experimentally are: Digitaria sanguinalis (L.) Scop., Digitaria violascens (L.) Link, Echinocloa colonum (L.) Link, Echinocloa crusgalli (L.) Beauv., Eleusine indica (L.) Gaertn., Erianthus giganteus (Walt.) Muhl, Lamarckia aurea (L.) Moench, Narenga porphyrocoma (Hance) Bor., Panicum dichotomiflorum Michx., Paspalum bosianum Flugge, Paspalum fimbriatum H. B. K., Paspalum virgatum L., Setaria lutescens (Weigal) F. T. Hubb, Setaria magna Griseb, Setaria poiretiana (Schult.) Kunth, and Setaria verticulatta (L.) Beauv. Anzalone (7) in 1963 found four cultivated varieties of rice (Oryza sativa L.) susceptible to strain H of SCMV. Todd (79) in 1964 reported St. Augustine grass (Stenotaphrum secundatum (Waltz.), Kuntze) susceptible to SCMV. Also, in 1964 Abbott and Tippett (4) using four strains of SCMV, found Andropogon virginicus L., Sorghum halepense (L.) Pers., Triticum aestivum L., Secale cerale L., and Hordeum vulgare L. susceptible to SCMV. Recently, Perdomo and Forbes (55) found Raoul grass (Rottboellia exaltata L. F.) susceptible to SCMV.

SCMV is transmitted mechanically and by several aphid species (74). The virus has a stylet-borne relationship with aphid vectors. Brandes (17) in 1920 demonstrated that Rhopalosiphum maidis Fitch was able to transmit the virus to healthy plants after a feeding period on infected plants. R. maidis was the only known vector of SCMV until 1933 when Ingram and Summers (41), in preliminary experiments, showed that the rusty plum aphid, Hysteroneura setarie Thos. was also capable

of transmitting the virus. Ingram and Summers (42) in 1938 reported that Toxoptera graminum Rond. could also transmit the virus. Tate and Vandenburg (75) in 1939 reported Carolinaia cyperi Ainslie as a vector in Puerto Rico. Recently, other aphids have been shown to transmit SCMV. They are: Acyrthosiphon pisum Harr. (3), Dactynotus ambrosiae Thos. (3), Amphorophora sonchi Destl. (3), and Myzus persicae Sulz. (8).

Partially purified preparations from mosaic infected sugarcane and corn yielded rod-shaped virus particles which had an average diameter of 15 mu and a length of 620-670 mu (57, 66). Herold and Weibel (38) reported rod-shaped virus particles averaging 760 $\pm$ 10 mu in length and 12-13 mu in diameter from leaf dip preparations. Pirone and Anzalone (57), using the concept of normal length as set forth by Brandes and Wetter (19), found that partially purified preparations from juice of mosaic infected sorghum yielded long flexuous rod-shaped virus particles with a normal length of 755 mu. The fact that SCMV particles have normal lengths of approximately 750 mu places the virus in the potato virus Y (PVY) group in Brandes' (18) system of classification.

Several strains of SCMV have been described (2, 74). These strains were identified according to their reaction on certain differential varieties (5, 74). Tims and Edgerton (77) were the first to mention the possibility of strains of the virus. This hypothesis was based on the differences in degree of infection with mosaic, observed in four varieties of sugarcane at two localities in Louisiana. Storey (71) in 1927 reported that he was able to separate

two supposed strains of SCMV. The identification of these strains was based upon differences in regional distribution and host range in Natal, South Africa. Tims, Mills, and Edgerton (78) in 1935 reported differences in virulence between the viruses from areas of heavy and light mosaic incidence. They concluded that "two very distinct types of mosaic, recognized by very distinct symptoms, occur in Louisiana." Summers (72) also in 1935 described four strains which were designated 1, 2, 3, and 4. These strains were differentiated principally by symptoms produced on the sugarcane variety C.P. 28-60. He later reported (73) seven strains which were designated as A, B, C, D, E, F, and G, and three substrains of D, with strains A, B, C, and D corresponding to the previously designated 1, 2, 3, and 4. The differentiation of these strains was based on symptoms produced on the sugarcane varieties, C.P. 31-294, C. P. 29-291, and Co. 281.

Summers, Brandes and Rands (74) in 1948 explained in detail the experiments that led to the differentiation of the 10 strains and substrains and furnished a key for their identification on Summers' differential host varieties.

Liu (46) in 1950 described four strains of SCMV in Taiwan, designating them A, B, C, and D. Since the differential hosts used were different from those used by Summers, no comparison can be made between these strains and those described by Summers. Later, Liu and Li (47) reported the existence of only three strains of SCMV in Taiwan. These were designated as "short stripe type (SS)," "yellow-stripe type (YS)," and "fine-stripe type (FS)."

Abbott (2) in 1961 reported a new strain of SCMV designated as strain H. This strain is considered to be the most severe strain due to its ability to attack several varieties of sugarcane previously considered immune to mosaic.

Abbott and Tippett (5) in 1966 reported the results of a study of Summers' stock cultures of five strains and four substrains on various differential hosts. These authors reported that strains A, B, D, and H could be differentiated using the differential host varieties C.P. 31-588 and C.P. 31-294. In this study, Co. 281 was excluded because it differentiates only strain C which is rare. According to these workers, this strain can be identified in the field without transfer to differential hosts.

In 1963, a new mechanically transmissible virus was isolated from corn in Ohio (84). Since then, a similar virus disease has been reported from a number of states (24, 58, 69). This virus disease has been called maize dwarf mosaic virus (85) because of the dwarfing symptoms which it supposedly produces on corn. Williams and Alexander (85) reported that MDMV had properties similar to sugarcane mosaic virus, although, no relationship was shown in their preliminary serological tests. Recently, Shepherd (66) and Bancroft et al. (11) have shown that MDMV and SCMV are morphologically similar and serologically related. Wagner and Dale (83) tested several isolates of MDMV from several states and found that all were serologically related to SCMV. These authors have suggested that MDMV is probably a strain of SCMV. This virus, however is unique in that it readily infects Johnson grass (Sorghum halepense) (84). For this reason, it is

sometimes referred to as the Johnson grass strain of SCMV (68). Sugarcane is highly refractory to infection by this strain (25).

A number of workers have investigated the physical properties of SCMV (1, 5, 6, 22, 23, 45). However, the data presented is very inconsistent. Chona (22) in 1944 reported thermal inactivation points of 45, 55, and 65°C, respectively, for the three strains with which he worked. Adsuar (6) reported a thermal inactivation point of 55°C. Costa and Penteado (23) also reported a thermal inactivation point of 55°C. Abbott (1) in 1953 reported that all strains of SCMV were inactivated at 53°C. Recently, Abbott and Tippett (5) reinvestigated the physical properties to determine if physical properties might supplement macroscopic symptoms in strain differentiation. In this study, thermal inactivation points were determined for three strains and eight variants of SCMV. The thermal inactivation points for strains A, D, and H were 53, 52, and 49°C, respectively. However, the fact that inactivation was obtained at one temperature and regained at a higher temperature might lead one to question the validity of these results.

A wide range of values has also been reported for the dilution end point (DEP) of SCMV (1, 5, 23, 45, 63). Rafay (63) in 1935 reported a dilution end point of 10^{-1} for SCMV. Lawas and Fernandez (45) in 1949 also reported 10^{-1} as the dilution end point. Costa and Penteado (23) reported a value of 10^{-5} for juice extracted from corn infected with SCMV. In 1953, Abbott (1) reported a dilution end point of 10^{-3} for six strains which he studied. Recently, Abbott and Tippett (5) made dilution end point studies of strains A, D,

and H. They reported values of 10^{-3} for strains A and D and 10^{-2} for strain H.

Desai (27) and Perez and Adsuar (56) were the first to show that SCMV is antigenic. Perez and Adsuar suggested the possibility of using the precipitin reaction in testing for relationships among strains of SCMV. Recently, Abbott and Tippett (5) concluded that differentiation of strains of SCMV on the basis of symptoms and physical properties was limited, and suggested that serology might be of value in strain differentiation. Until recently, however, this has not been feasible due to the absence of a suitable method of purification. Pirone and Anzalone (57) and Shepherd (66) have purified SCMV, and have obtained antisera of sufficiently high titer for use in serological studies.

Serology has proven useful in establishing relationships of many plant viruses (11, 31, 34, 65, 86). Bennett (13) in 1953 reported that serology was more accurate than differential hosts and physical properties in differentiating strains of most plant viruses. According to Ball (10), Dvorak was the first to apply serological methods to plant viruses. Purdy (62) in 1928, using precipitin and complement fixation tests, showed the specificity of serological reactions. Birkeland (15) was the first to show that strains of plant viruses contained specific antigens which differentiated them from other members of the group. Chester (21) in 1936 used the cross absorption technique to demonstrate serological differences among strains of the same virus. Despite these findings, the difficulty in obtaining antisera of high titer, and the large amounts of antigen

required for available serological techniques limited the use of serology in studies of virus relationships. In recent years, the development of new serological techniques has made serology a more useful tool for determining virus relationships (29, 54, 82).

Microprecipitin tests have been widely used in serological studies of plant viruses. Microprecipitin tests require only small amounts of antigen and antisera (9). Scott et al. (65), using the microprecipitin test, found that bean pod mottle and red node viruses were not related. Shepherd (66), and Bancroft et al. (11) found that MDMV was serologically related to SCMV using the microprecipitin test.

The Ouchterlony agar double diffusion test represents one of the newest serological techniques. Van Slogteren (82) was the first to apply this procedure to plant viruses. Scott et al. (65) showed that bean pod mottle and red node viruses were serologically unrelated, each giving distinct lines of precipitation. Willison et al. (86) used agar double diffusion tests to show the relationship of certain stone fruit viruses. Grogan and Kimble (34) using this procedure were able to show that severe bean mosaic virus (SvBMV) from Mexico, southern bean mosaic virus (SBMV) and its related strain in cowpea were serologically related, but not identical. Recently, Fulton (31) used agar double diffusion tests to identify and show relationships of certain stone fruit viruses.

The Ouchterlony double diffusion tests are well adapted for use with the spherical and shorter rod-shaped plant viruses, however, the long flexuous rod-shaped plant viruses do not diffuse well in agar gels. Purcifull and Shepherd (61) have shown that the flexuous

rod-shaped viruses could be degraded with alkaline buffers into antigenically active fragments suitable for use in agar gels.

Biological properties are useful in identifying and grouping viruses. Such things as host range, manner of transmission (mechanical, insects, and by nematodes and soil fungi) are useful in establishing relationships.

There are several plant viruses which are known to be soil transmissible. Harrison (36) defined soil-borne viruses as those "with an underground method of natural spread which does not depend simply on contact between tissues of infected and healthy plants." According to Harrison (37), Beijerinck in 1898 showed that tobacco seedlings became infected with tobacco mosaic virus (TMV) when grown in soil in which diseased tobacco plants had been grown. However, McKinney (50) is credited with establishing the fact that some viruses infect plants naturally through their roots. McKinney (52) speculated that wheat mosaic virus (WMV) might be transmitted by nematodes, soil-borne insects, fungi, or without a vector from soil or organic particles. Other virus diseases which were shown to be transmitted by growing plants in infested soil were; tobacco rattle virus (37), grapevine fan-leaf (37), lettuce big-vein (43), and tobacco necrosis virus (70). However, not much attention was given to soil-borne viruses until 1958, when Hewitt et al. (39) showed that grapevine fan-leaf virus was transmitted by the plant parasitic nematode, Xiphinema index. Also in 1958 Fry (30), and Grogan et al. (33) associated Olpidium brassicae, a fungus, with the transmission of lettuce big vein virus. In 1960, Olpidium was associated with tobacco stunt virus (40), and with

tobacco necrosis virus (76). Recently, more convincing evidence has been presented concerning the transmission of these viruses by Olpidium (35). Brakke and Estes (16) have recently shown a correlation between the presence of Poloymyxa graminis and the transmission of soil-borne wheat mosaic virus. Barley yellow mosaic virus (BYMV) (53) and oat mosaic virus (OMV) (20) are also thought to be transmitted in a manner similar to soil-borne wheat mosaic (35).

Some viruses have been reported to be soil-borne in the absence of a biological vector. Such viruses are; tomato bushy stunt virus (48), tobacco mosaic virus (35), and chlorotic streak of sugarcane (14). Several workers have advanced the hypothesis that soil transmission could occur through wounds produced by roots growing through soil or sand. Miyamoto (53) proposed that WMV and BYMV could survive on soil particles and infect cereal hosts without a vector. However, Grogan and Campbell (35) state that "it is practically impossible to maintain freedom from a fungus, such as Olpidium, under ordinary greenhouse conditions." Thus, Grogan and Campbell are of the opinion that accidental contamination could account for the reported transmissions in the absence of a biological vector.

MATERIALS AND METHODS

I. Virus Strains and Source Plants

Strains A, B, D, and H of SCMV used in this study were obtained from the collection maintained at the U. S. Sugarcane Field Station, Houma, La. Beefbuilder T sorghum (Sorghum vulgare x Sorghum vulgare var. sudanense) was used as the source of the virus. Mechanical inoculations were made with freshly expressed sap by means of a gauze pad onto leaves that had been dusted with 600 mesh carborundum. Source plants were then placed in the greenhouse.

II. Virus Assay

Since there are no local lesion hosts known for SCMV, the per cent infectivity test was employed for assaying the virus. Unless otherwise noted, dilutions of 10^{-0}, 10^{-1}, 10^{-2} and 10^{-3} were used for assaying the virus. Each dilution was assayed by mechanically inoculating 50 sorghum seedlings. Following inoculation, the seedlings were returned to the greenhouse for symptom development.

III. Differential Varieties

Differential sugarcane varieties used by other workers (5, 74) were obtained from the U. S. Sugarcane Field Station, Houma, La., and inoculation for comparison with the results of these studies. Thirty-five eyes each, of the differential varieties C.P. 31-294 and C.P. 31-588 as well as P.O.J. 234 were planted in the greenhouse.

SCMV strains A, B, D, H, and the Johnson grass strain were inoculated at the two-leaf stage into 6 or 7 plants each of the two differential varieties, as well as into P.O.J. 234.

IV. Symptoms on Sorghum Produced by the Different Strains

Initial symptoms of strains A, B, D, and H on beefbuilder sorghum were similar to the symptoms described by Edgerton (28). However, soon after initial symptom expression, a severe leaf necrosis often developed on the leaves of infected sorghum plants. The leaf necrosis appeared to be most prevalent under conditions of low temperature and high humidity. Experiments were made to determine the relationship of the leaf necrosis to the four strains of SCMV. Ten beefbuilder sorghum seedlings were inoculated with strains A, B, D, and H. The inoculum used was standardized by increasing in sorghum for three successive generations. After inoculation, the plants were placed in a Sherrer-Gillete controlled environment chamber for symptom development. The environment of the chamber consisted of an air temperature of 85°F, 2000 ft. candles of light.

V. Physical Properties of Strains of SCMV

A. Thermal inactivation point (TIP)

Infected tissue of each strain which had been increased in sorghum for 15 days was harvested and ground in a fruit grinder. One ml aliquots of the undiluted juice were placed in 5 ml serological tubes which had been preheated. Sterile 1 ml syringes were used to place the material into the tubes with care taken to avoid splashing material onto the sides of the tubes. The tubes were immersed in a continuously

agitated water bath for 10 min at 49, 51, 53, 55, and 57°C, respectively. The tubes were then cooled, and each sample was assayed on 40 sorghum seedlings.

B. Dilution end point (DEP)

Dilution end points were determined for each strain of SCMV. The inoculum used in these tests was standardized by increasing the sorghum for three successive generations. Infected tissue of each strain was harvested for 15 days, weighed into 2 gm samples, and ground in a mortar. Aliquots of freshly expressed juice were diluted 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Each dilution was assayed on 100 sorghum seedlings.

VI. Comparison of Three Methods of Virus Purification

Three methods of purification were compared to determine which yielded the greatest amount of infectious virus. Strain H was increased in sorghum for 3-4 weeks. The tissue was chopped and divided into 3 aliquots. Each aliquot was purified by a separate technique. Assays were made to compare infectivity of the virus at various stages of each procedure. The procedures used were as follows:

1) Pirone and Anzalone (57) described a method for purifying SCMV from infected sorghum tissue (acid clarification method). One hundred and fifty gm of sorghum tissue, collected 3 weeks after inoculation, was blended in a Waring blendor with an equal volume of .02 M sodium sulfite. The supernatant was acidified with 1.0N HCl to pH 4.7 and centrifuged in a Sorvall RC-2 centrifuge for 5 min at 5000 rpm. The supernatant was then centrifuged at 27,000 rpm for

5 min using a number 30 rotor in a Spinco L-2 centrifuge. The resulting supernatant was centrifuged at 30,000 rpm for 1.5 hr. The pellets were pooled and resuspended in 2 ml of .02M sodium sulfite for 2-3 hr, using a magnetic stirrer. The preparation was then centrifuged for 5 min at 5000 rpm, and the supernatant was centrifuged for 1 min at 4000 rpm in a number 50 Spinco rotor using 3-ml tubes and adapters. The supernatant was layered onto a density-gradient column prepared in 1.25 x 3.5 inch tubes by layering 10, 14, 14, and 14 ml of 10, 20, 30, and 40% sucrose, respectively, in 0.02M sodium sulfite, and centrifuged in an SW 25.2 rotor at 24,000 rpm for 1.5 hr. The virus band was removed with an ISCO density-gradient fractionator.

Infectivity assays at each step in the purification procedure were made by inoculating 50 sorghum seedlings with undiluted and with 10^{-1} and 10^{-2} dilutions of the virus.

2) Shepherd (66) described a method for the purification of a mosaic virus of corn in California (chloroform-strong buffer clarification method). One hundred and fifty gm of sorghum tissue, collected 3 weeks after inoculation, was blended in a Waring blender with an equal volume of 0.5M sodium citrate containing 0.5% mercaptoethanol. The juice was expressed through two thicknesses of cheesecloth. An equal volume of chloroform was added to the extract and the mixture was shaken, then centrifuged (10,000 rpm for 10 min) to recover the aqueous phase. This was followed by high speed centrifugation (30,000 rpm for 1.5 hr in a number 30 rotor of a Spinco L-2 centrifuge) of the clarified extract. Following high speed centrifugation, pellets were resuspended as before, except 0.005M borate, pH 8.2 was used as the

resuspending liquid. The preparation was then centrifuged for 5 min at 5,000 rpm. The supernatant was given a high speed centrifugation (1 min at 40,000 rpm) in a number 50 Spinco rotor using 3-ml tubes and adapters. The supernatant was layered onto sucrose density-gradients as before, except, the gradients were made in 0.005M borate, pH 8.7. The gradients were centrifuged at 25,000 rpm for 1.5 hr. The virus band was removed using an ISCO density-gradient fractionator. Infectivity assays were made at each step in the purification procedure.

3) Delgado-Sanchez and Grogan (26) described a method for the purification of potato virus Y from tobacco tissue (chloroform-water clarification method). This method was chosen for use with SCMV because of the similarity in morphology and physical properties of SCMV with other members of the potato virus Y group in Brandes' (18) system of classification. The method was modified for use with SCMV. One hundred fifty gm of infected sorghum tissue collected 3 weeks after inoculation, was blended with an equal volume of distilled water containing 0.3% ascorbic acid, .3% 2-mercaptoethanol and 0.01M sodium diethyldithiocarbamate (SDDC). The juice was expressed through two thicknesses of cheesecloth. An equal volume of chloroform was added to the extract and the mixture was shaken, then centrifuged (10,000 rpm for 10 min) to recover the aqueous phase. This was followed by high speed centrifugation (30,000 rpm for 1.5 hr in a number 30 Spinco rotor of a Spinco L-2 centrifuge) of the clarified extract. After high speed centrifugation, the pellets were resuspended in 0.1M borate, pH 8.2 containing 0.01M ethylenediamine

tetra-acetic acid (EDTA). The resuspended material was centrifuged for 5 min at 5,000 rpm. The supernatant was given a high speed centrifugation (1 min at 40,000 rpm) in a number 50 Spinco rotor using 3-ml tubes and adapters. The supernatant was layered onto sucrose density-gradients as before, except, the gradients were made in 0.005M borate, pH 8.2. The gradients containing the virus were centrifuged for 1.5 hr at 25,000 rpm. The virus band was removed with an ISCO density-gradient fractionator. Infectivity assays were made at each step in the purification procedure.

VII. Serology of Strains of SCMV

A. Production of antisera

Antiserum to SCMV was prepared by intravenous and intramuscular injections (10) of partially purified virus into a rabbit. Virus used as antigen was obtained using the modification of Delgado-Sanchez and Grogan's procedure for potato virus Y. The virus was obtained directly from the density-gradient column. One ml of each of the four SCMV strains and the Johnson grass strain of SCMV was injected intravenously into rabbits at the first injection. Intramuscular injections of 0.5 ml of antigen and 0.5 ml of Freund's incomplete adjuvant were also administered the first week, and continued at weekly intervals for 5-6 weeks. Serum was obtained 7 days after the last injection. Dilution titration was used to determine the antiserum titers.

B. Microprecipitin tests

Microprecipitin reactions under mineral oil were used as one of the serological tests on the various sera and virus strains. All

antisera and virus dilutions were made in 0.85% saline. In these tests, antiserum end points were determined by mixing partially purified virus with an equal volume of antisera diluted in twofold series. The antigen-serum mixture was incubated overnight before observation of the final results.

C. Agar diffusion tests

SCMV, a long flexuous rod-shaped plant virus, will not diffuse in agar gels. However, Purcifull and Shepherd (61) devised a method for degrading flexuous rod-shaped virus particles into small antigenically active fragments which diffuse readily in agar gels. The method used in these studies was a modification of Purcifull and Shepherd's. About 150 gm of infected tissue of each strain of SCMV, including the Johnson grass strain of SCMV, was partially purified using the modification of Delgado-Sanchez and Grogan's method for potato virus Y. After clarification and high speed centrifugation, the pellets were resuspended in 2 ml of distilled water. The preparation was then centrifuged for 5 min at 5,000 rpm. The clarified supernatant of each strain was added to an equal volume of .1M ethanolamine, pH 10.5, in order to degrade the virus particles into antigenically active fragments (60).

Agar gel double diffusion tests were done in plastic petri dishes (Falcon Plastics, Division of B-D Laboratories, Los Angeles, Calif.). Eleven ml of 0.85% Ion Agar No. 2 (Consolidated Laboratories, Chicago Heights, Ill.) containing 0.85% sodium chloride and 0.4% sodium azide were poured into each standard 9 cm dish. Two patterns were used in

these tests. One pattern had a center well 7 mm in diameter surrounded by 8 peripheral wells of 4 mm diameter at a distance of 7 mm from the edge of the central one. All wells held approximately 0.2 ml. Antiserum was placed in the central well and the peripheral wells were filled with antigen. Another pattern consisted of a center well 7 mm in diameter surrounded by four equally spaced peripheral wells of 4 mm diameter at a distance of 7 mm from the edge of the central one. Antigen was placed in the central well and the outer wells were filled with the antisera. During formation of precipitin zones, the diffusion plates were kept at room temperature in a moist chamber. Zones were most easily observed with horizontal illumination against a black background.

VIII. Soil Transmission Studies

In the course of bioassays on strain H of SCMV, noninoculated sorghum seedlings often became infected when grown in greenhouse flats which contained infected seedlings. The frequent occurrence of the phenomenon under screened greenhouse conditions, in the absence of aphids, suggested that some method of spread other than by aphids might be responsible.

A. Seed transmission studies

Sorghum grains were germinated in greenhouse flats and allowed to grow for 1-2 months. Seedlings were examined periodically for mosaic symptoms.

B. Soil transmission studies

In initial tests, sorghum grains were planted in rows in greenhouse flats. At the two-leaf stage, alternate rows were inoculated with strain H of SCMV. Controls were sorghum plants grown in porcelain pans to keep their root systems separate from those of inoculated plants. The pans containing the control plants were placed in the center of a flat containing inoculated plants. This allowed the control plants to be near the inoculated plants in order to check for possible aphid transmission.

Preliminary tests indicated that root contact was not necessary in order to obtain transmission. Two types of tests were set up to test whether root contact was necessary for transmission. The first method, which will be referred to as Method A, consisted of placing porcelain pans containing noninoculated seedlings in flats as described previously. The pans were set below the soil level to allow water to run from the flat containing the infected plants into the pan containing the noninoculated plants. The controls were set up in the same manner except the porcelain pans were set well above the soil level. Care was taken to avoid water splashing into the controls when the infected plants were watered.

Another test (Method B) involved planting sorghum grains in 4-inch peat pots at either end of an 8" x 4" plastic container. Plants at one end of the container were inoculated with SCMV at the two-leaf stage, while the plants at the other end were left noninoculated. Since there was no soil between the peat pots, roots could be kept separate and under observation. The only contact

between the plants was by means of water at the bottom of the container.

Experiments mentioned previously (Method A and Method B) were also made under screened cages. The tests were set up as before under 32 mesh screen cages in a screened greenhouse. Cages were sprayed with phosdrin and plants were germinated under the cages and inoculated as in other tests.

EXPERIMENTAL RESULTS

I. Reaction of Virus Strains on Standard Differential Varieties

Table 1 shows the infection ratio when the standard differentials were inoculated with each virus strain. All strains of SCMV showed a high infection ratio. The differential varieties inoculated with the Johnson grass mosaic did not become infected.

Strain B could be differentiated from the other strains quite readily. Symptoms produced were typical of those described by Abbott and Tippett (5). The symptoms produced by strain D were also different from those produced by strains A, B and H. However, they were not typical of those described by Abbott and Tippett. Strains A and H produced similar symptoms on the differential varieties and could not be differentiated.

II. Symptoms on Sorghum Produced by the Different Strains

The leaf necrosis developed on plants infected with strains A, D, and H, but not with strain B (Plate 1). Necrosis was most severe on plants infected with strains A and H.

III. Physical Properties of Strains of SCMV.

A. Thermal inactivation points

Thermal inactivation studies were made to determine if a difference existed among strains of SCMV in their tolerance to heat. The data in Table 2 show the results of three experiments. All strains of SCMV were active at 55°C, but inactive at 57°C.

Table 1. Results of inoculation experiments showing infection ratio of strains A, B, D, H, and Johnson grass mosaic in standard differentials.

Differential variety	Strain				
	A	B	D	H	J[b]
P.O.J. 234	7/7[a]	7/7	4/7	7/7	0/5
C.P. 31-294	7/7	5/7	2/7	7/7	0/6
C.P. 31-588	7/7	6/7	6/7	6/6	0/6

[a]Denominator, number of plants inoculated; numerator, number of plants infected.

[b]Johnson grass mosaic.

Plate 1. Reaction of beefbuilder sorghum to strains A, B, D and H of sugarcane mosaic virus (SCMV) when grown under conditions of low temperature and high humidity. Left to right strains A, B, D, and H.

Table 2. Thermal inactivation points of strains A, B, D, and H of SCMV in sorghum.[a]

Virus strain	Temperature °C.				
	49°	51°	53°	55°	57°
Strain A	78[b]	73	5	1	0
Strain B	63	38	13	5	0
Strain D	98	53	12	1	0
Strain H	98	35	18	9	0

[a]Results of three experiments.

[b]Data expressed as per cent of plants infected.

B. Dilution end points

Dilution end point studies were made to determine if differences existed among strains in their tolerance to dilution. The results of three experiments are shown in Table 3. The data indicate a difference among the strains in their tolerance to dilution. Strains A and H were still infectious at 10^{-4}, strain D at 10^{-3}, and strain B at 10^{-2}.

IV. Comparison of Three Methods of Virus Purification

Preliminary attempts to purify the four strains by the acidification method indicated that strain B could not be purified by this technique. A comparative study was made of three purification methods to determine which procedure would give the highest yield of infectious virus, and which could be used for all strains. Assays were made of aliquots taken at different steps in each procedure. The steps which were assayed are shown in Table 4. Table 5 shows the results of the three methods of purification.

With each procedure, the virus banded in the gradients in a distinct zone about 22 mm from the miniscus. As a measure of purity, ultraviolet absorption spectra of the material taken from the gradients were determined. A typical ultraviolet absorption spectrum was not obtained with material resulting from the acid clarification method, nor from the chloroform-buffer clarification method. However, an absorption spectrum typical for nucleoproteins, with the absorption maxima and minima near 260 and 240 mu, $E\frac{260}{280} = 1.24$ and $E\frac{max}{min} = 1.06$, was obtained with material purified with the chloroform-water clarification method (Figure 1).

Table 3. Dilution end point of strains A, B, D, and H of SCMV in sorghum.[a]

Virus strain	Dilution			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Strain A	39[b]	14	8	1
Strain B	25	<1	0	0
Strain D	50	7	3	0
Strain H	33	8	4	1

[a]Results of three experiments.

[b]Data expressed as per cent of plants infected at each dilution.

Table 4. Procedures used in purification by different methods after which assays were made.

Treatment	Procedure		
	Acid clarification	Chloroform-buffer	Chloroform-water
1) Homogenization	.02M Na_2SO_3	0.5M Na citrate, 0.5% mercaptoethanol	0.3% Ascorbic acid, 0.01M sodium diethyldithio carbamate, 0.3% 2-mercaptoethanol
2) Clarification	pH adjusted to 4.7, low speed (5,000 rpm 5 min) high speed (5 min 27,000 rpm)	Chloroform, low speed (10 min 10,000 rpm)	Chloroform, low speed (10 min, 10,000 rpm)
3) Resuspended pellet after 1.5 hr	Resuspended in .02M Na_2SO_3	Resuspended in 0.005M borate, pH 8.2	Resuspended in 0.1M borate 0.01M EDTA, pH 8.2
4) Low speed	5 min at 5,000 rpm, 1 min at 40,000 rpm	same	same
5) Density gradient centrifugation	1.5 hr at 25,000 rpm	same	same

Table 5. Comparative infectivity of SCMV after treatments listed in Table 3.[a]

Treatments	Procedure		
	Acid clarification	Chloroform-buffer	Chloroform-water
1) Homogenization	42	19	38
2) Clarification	8	3	33
3) Resuspension	2	4	20
4) Low speed	10	3	10
5) Density gradient	2	2	15

[a]Average of two experiments. Data expressed as per cent of plants infected at a dilution of 10^{-1}.

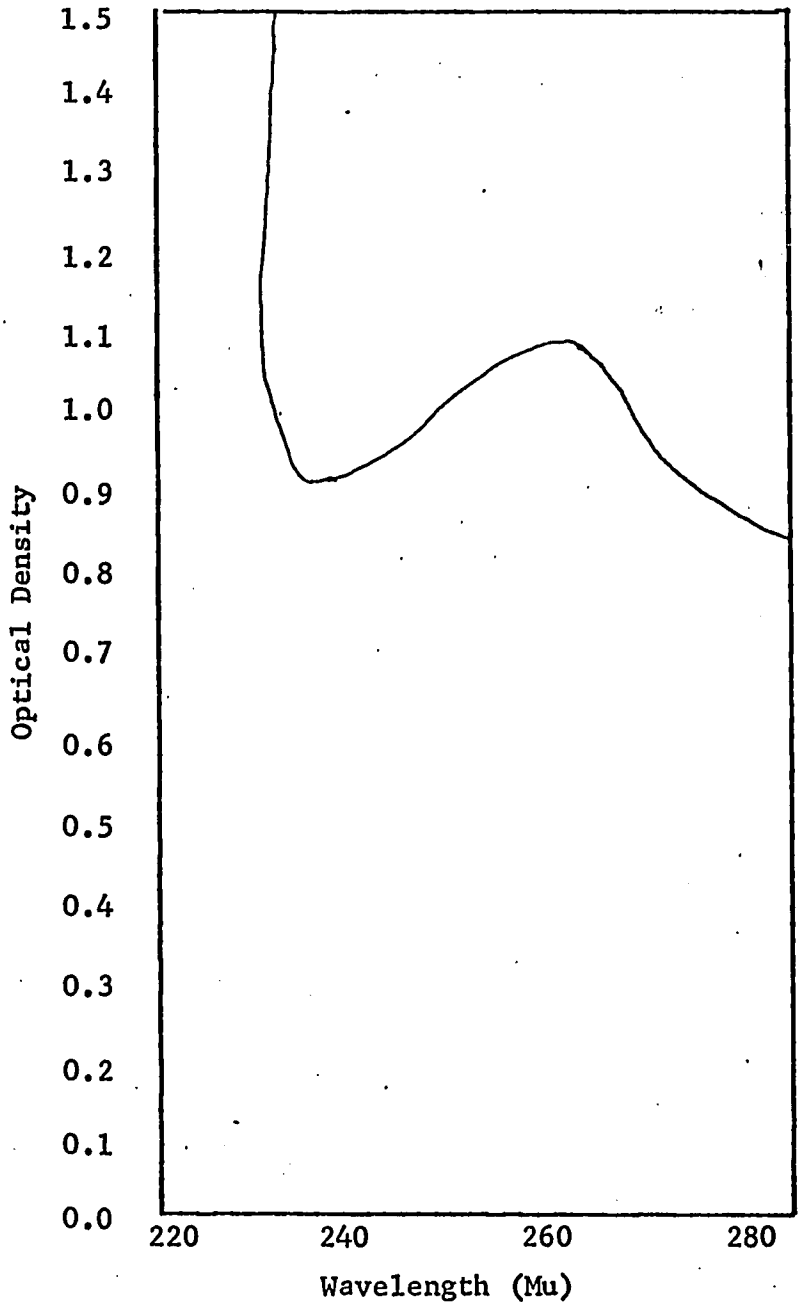


Figure 1. Absorption spectrum of a purified preparation of sugarcane mosaic virus. The curve is a tracing using a Perkin Elmer Spectrophotometer.

V. Usefulness of the Three Procedures for Purification of Different Strains

Using the scanning patterns from the density-gradient fractionator as a measure of the relative virus yields, a difference was noted with several of the strains in their reaction to the three methods of purification. Strains A, D and H gave relatively high yields with each purification procedure. Numerous attempts to purify strain B and the Johnson grass strain with the acid clarification method failed. However, both strain B and the Johnson grass strain gave relatively high yields of virus when purified with either the chloroform-strong buffer clarification method, or the chloroform-water clarification method.

VI. Serology of Strains of SCMV

A. Microprecipitin tests

Antisera to strains A, B, D, and H were prepared and tested with the respective homologous and heterologous antigens. In addition, the Johnson grass strain of SCMV present in Louisiana was tested against antiserum to strain H to determine its relationship to SCMV. Healthy sorghum protein tested against all antisera served as the control. The precipitates obtained with the antisera and the partially purified viruses were of the open flocculent type characteristic of rod-shaped plant viruses.

The precipitin end points with antisera to strains A, B, D, and H were 1/128, 1/256, 1/128, and 1/256, respectively, when antisera reacted with the homologous antigens (Table 6). The heterologous precipitin end point showed slight variations, but never varied more

Table 6. Microprecipitin reactions of sugarcane mosaic virus (SCMV) strains against antisera to strains A, B, D, and H.

Antigen	Antiserum to:	Relative amounts of precipitate with various serum dilutions (as reciprocals)[a]								
		Antiserum							Normal serum	
		8	16	32	64	128	256	512	0	4
St. A-SCMV	St. A-SCMV	++	++	++	+	+	-	-	-	-
"	St. B- "	++	++	++	++	+	+	-	-	-
"	St. D- "	++	++	++	+	+	-	-	-	-
"	St. H- "	++	++	+	+	+	+	-	-	-
St. B-SCMV	St. B-SCMV	++	++	++	++	+	+	-	-	-
"	St. A- "	++	++	++	+	+	-	-	-	-
"	St. D- "	++	++	+	+	+	-	-	-	-
"	St. H-"	++	++	++	+	+	+	-	-	-
St. D-SCMV	St. D-SCMV	++	++	++	+	+	-	-	-	-
"	St. A- "	++	++	++	+	+	-	-	-	-
"	St. B- "	++	++	++	++	+	+	-	-	-
"	St. H- "	++	++	++	++	+	+	-	-	-
St. H-SCMV	St. H-SCMV	++	++	++	++	++	+	-	-	-
"	St. A- "	++	++	++	+	-	-	-	-	-
"	St. B- "	++	++	++	++	+	+	-	-	-
"	St. D- "	++	++	++	++	++	+	+	-	-
JGM-St.SCMV	St. H-SCMV[b]	++	++	+	+	-	-	-	-	-
Healthy sorghum protein	St. A-SCMV	+	-	-	-	-	-	-	-	-
"	St. B- "	+	-	-	-	-	-	-	-	-
"	St. D- "	+	-	-	-	-	-	-	-	-
"	St. H- "	+	-	-	-	-	-	-	-	-

[a] ++ = Maximum precipitate; - = no precipitate. [b] Johnson grass strain of SCMV.
[c] Data shown is an average of three experiments.

than one dilution from the homologous reaction, except the heterologous reaction involving strain H antigen and strain A antiserum. The Johnson grass strain gave a precipitin end point of 1/64 when reacted against strain H antiserum. The antisera reacted with healthy sorghum protein up to 1/8. However, this precipitate was easily distinguished from that produced by the virus. There was no reaction of the viruses against normal serum.

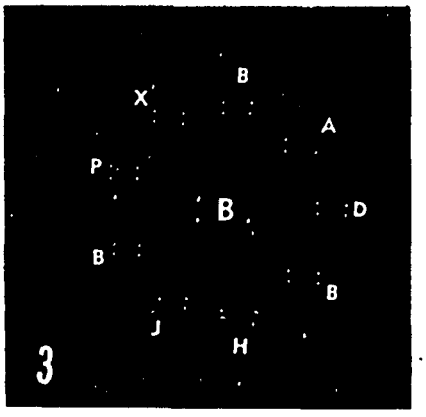
These results show that strains A, B, D, and H are closely related serologically and cannot be differentiated in microprecipitin tests. These results also show that the Johnson grass strain of SCMV in Louisiana is serologically related to SCMV, but not as closely as the strains are to each other.

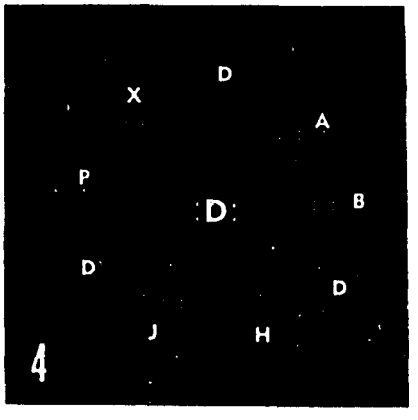
B. Agar diffusion tests

Figures 2-10 show the results of agar diffusion tests. When the antisera of strains A, B, D, and H were tested against respective homologous and heterologous antigens, all antisera reacted against antigens of strains A, D and H (Figures 2-10). The zones produced by these antigens banded together indicating that they were closely related serologically. Antigen of strain B did not react against any of the antisera, including its own, indicating that it was probably present in too low a concentration to detect. Antigen of Johnson grass mosaic gave a very weak reaction against antisera to strains of SCMV and spurring occurred (Figure 10).

Figures 2-5. Agar diffusion tests with strains of SCMV. 2) Precipitin bands produced between peripheral wells containing degraded virus protein of strains A, B, D, H and Johnson grass mosaic and healthy sorghum protein and the center well containing antiserum against strain A. 3) Center well containing antiserum against strain B. 4) Center well containing antiserum to strain D. 5) Center well containing antiserum against strain H.


A
X
B
P
A
D
A
J
H
2


B
X
A
P
B
D
B
B
J
H
3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X
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B
D
D
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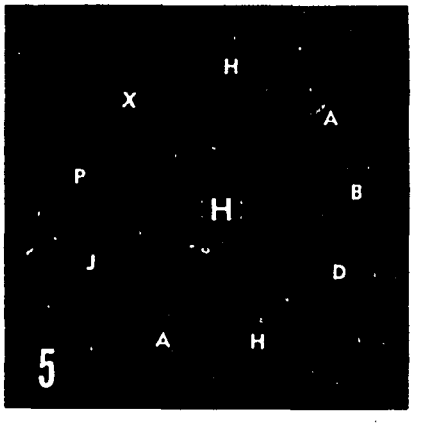
H
4


H
X
A
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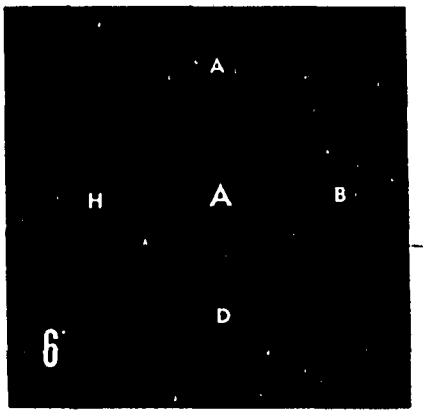
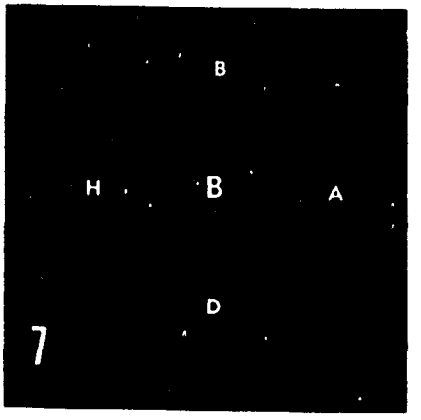
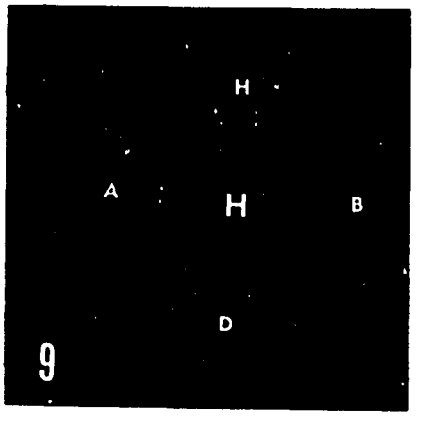
J
D
A
H
5

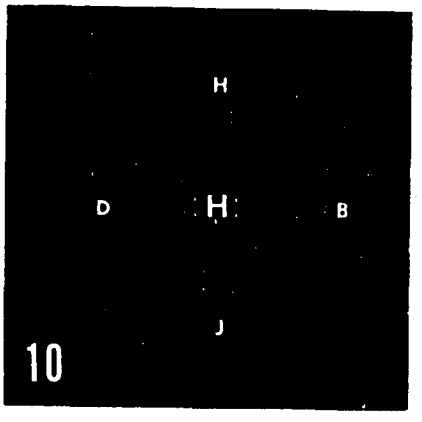
Figures 6-10. Agar diffusion tests with strains of SCMV. 6) Precipitin bands produced between peripheral wells containing antisera against strains A, B, D, and H and Johnson grass mosaic and the center well containing degraded virus protein of strain A. 7) Center well containing degraded virus protein of strain B. 8) Center well containing degraded virus protein of strain D. 9) Center well containing degraded virus protein of strain H. 10) Center well containing degraded virus protein of strain H.


A
H
A
B
D
6


B
H
B
A
D
7


D
H
D
A
B
8


H
A
H
B
D
9


H
D
H
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10

VII. Soil Transmission Studies

A. Seed transmission

Preliminary tests indicated that SCMV was being transmitted from infected plants to noninoculated plants in the absence of an aerial vector. Experiments were made to determine if the virus was being transmitted through the seed.

A total of 1168 sorghum seedlings were examined for possible seed transmission. Of the seedlings tested, no infected plants were obtained.

B. Soil transmission

1. Initial transmission tests

Seed transmission tests indicated that the virus was not being transmitted through the seed. Tests were then made to determine if the virus was being transmitted through the soil in some manner. Initial tests were designed to show that the virus was being transmitted through the soil. Initial transmission tests were repeated nine times.

The data in Table 7 show the results of these tests. In the first five experiments, transmission ranged from .7 to 5.4%. Nine to twenty days were required for transmission, with nine days being the shortest period of time in which transmission was obtained. In the first five experiments, two plants became infected in the controls. It seemed possible that this was due to contamination since no attempt was made to avoid water splashing into the pans containing the control plants. In the last four experiments, care was taken to avoid

Table 7. Transmission of sugarcane mosaic virus (SCMV) from infected plants to adjacent noninoculated sorghum plants.

Test	Test Plants		%	Check Plants	
	Exposed	Infected	Transmission	Exposed	Infected
1	201	11	5.4	138	0
2	288	8	2.7	141	1
3	318	13	4.1	60	0
4	273	10	3.7	138	1
5	286	2	.7	185	0
6	254	2	.8	193	0
7	288	9	3.1	141	0
8	200	10	5.0	154	0
9	399	18	4.5	702	0

water splashing into the controls. In these experiments, transmission to the test plants ranged from .8 to 5%. There was no transmission to the controls.

2. Transmission in the absence of root contact

The fact that contamination occurred in the controls suggested that root contact was possibly not necessary in order to obtain transmission. To determine if root contact was necessary, two types of tests (Method A and Method B) were made.

The data in Table 8 show the results of Method A. Transmission to plants grown in soil adjacent to inoculated plants was 4.5 to 8.8%. Transmission to plants grown in porcelain pans into which water was allowed to wash was 6.2%. In pans which were watered separately, there was no transmission. Root contact was absent in both cases.

Data in Table 9 show the results of Method B. Transmission was about 5% in all replications. There was no transmission to the controls.

3. Transmission under screen cages

Tests using methods A and B were made under 32 mesh screen cages in a screened greenhouse. Data in Table 10 show that transmission ranged from 1.6 to 10.1% with Method A. Transmission ranged from .9 to 2.5% with Method B.

4. Identity of the virus being transmitted.

Serological, and infectivity tests and EM preparations of leaf dip preparations confirmed that the virus being transmitted through the soil was SCMV.

Table 8. Transmission of sugarcane mosaic virus (SCMV) from infected plants to noninoculated sorghum plants through soil water (Method A).

	Transmission to	
	Plants Outside Pan	Plants Inside Pan
Soil Water Allowed to Wash into Pan	22/248 (8.8%)	10/160 (6.2%)
Soil Water Kept Out of Pan	20/442 (4.5%)	0/203 (0%)

Table 10. Transmission of sugarcane mosaic virus (SCMV) in sorghum plants under screened cages in the absence of root contact.

Method of avoiding root contact	Rep	Plants		% Transmission
		Exposed	Infected	
A	1	59	6	10.1
	2	72	6	8.3
	3	68	3	4.4
	4	66	1	1.6
B	1	226	2	.9
	2	232	6	2.5

5. Attempts to determine factors associated with transmission.

In attempts to correlate certain factors with transmission, sorghum grains were planted in steamed soil which had been autoclaved for 8 hr. In some instances, the sorghum grains were surface sterilized prior to planting. In all tests, a severe root and lower stem necrosis developed. Similar symptoms were observed when surface sterilized seed were germinated in sterile distilled water, and on water agar and potato dextrose agar (Plate 2). Of several varieties of sorghum tested, all produced the severe root necrosis. Transmission was generally correlated with the severity of the root necrosis.

Nematodes were detected in soil water extracts, however, none of these were plant parasitic species.

Attempts to isolate fungi or to detect them in the thin sections were negative. Bacteria were isolated in some instances, but it was impossible to determine their pathogenicity since the necrosis occurred spontaneously, even in seed which had been surface sterilized.

Plate 2. Necrosis on roots of surface sterilized sorghum seeds germinated in sterile water.

DISCUSSION

Since 1926 when McKinney (51) first demonstrated the existence of strains of tobacco mosaic virus (TMV), every virus that has been studied in detail has been found to exist in a range of forms or strains. Virus strains have been identified using a wide range of criteria such as reaction of differential host varieties, physical, chemical, and biological properties, particle length, and morphology.

Several strains of sugarcane mosaic virus have been described (2, 74). Summers (72, 73), and Summers, Brandes and Rands (74) differentiated strains of SCMV on the basis of their reaction on certain differential host varieties. The characters used as a basis for strain differentiation were: (1) nature of chlorosis, (2) presence or absence of necrosis in the lesions, (3) leaf sheath discoloration, (4) growth retardation, (5) germination recovery, (6) relative infectiousness, and (7) length of incubation period. Although a number of strains were differentiated by these workers, only four strains (A, B, D, H) have been identified in Louisiana since 1950 (5). Recently, Abbott and Tippett (5) reported that these strains could be differentiated on the differential host varieties C.P. 31-294 and C.P. 31-588 using the type of mosaic pattern as the principal diagnostic character. According to Price (59), unless the environmental conditions are carefully controlled it is difficult to compare symptoms.

In the experiments reported in this dissertation, in which each of the four strains was introduced into the standard differentials, only strain B showed symptoms similar to those described by Abbott and Tippett (5). Strain D could also be differentiated from the other strains, but symptoms were not typical of those described by Abbott and Tippett. Strains A and H showed similar symptoms on the differential varieties and could not be differentiated. Since there has been no attempt to describe symptoms under carefully controlled conditions, symptom expression should be interpreted with caution.

I. Physical Properties of Strains of SCMV

Thermal inactivation studies showed that all strains of SCMV were infectious at 55°C, but not at 57°C. These results agree with Adsuar (6), and Costa and Penteado (23) who reported the same TIP. These results do not agree with those of Chona (22), Abbott (1), and Abbott and Tippett (5). Chona reported TIP's of 45, 55, and 65°C, respectively, for three collections of SCMV in India. However, the figure of 65°C is much higher than that reported by other investigators (1, 6, 23). Abbott (1) in 1953 reported that all strains of SCMV were inactivated at 53°C. In a recent study, Abbott and Tippett (5) reinvestigated the physical properties of three strains (A, D, H) and eight variants of these strains. The TIP's for strains A, D and H were 53, 52, and 49°C, respectively. However, the fact that inactivation was obtained at one temperature and activity regained at a higher temperature leads this author to question the validity of such results.

Dilution end point studies indicate a difference among the four strains of SCMV in their tolerance to dilution. Strains A and H retained infectivity at dilutions of 10^{-4}, D at 10^{-3} and B at 10^{-2}. The relative concentration of strain B is 100 fold less than D. Thus, it appears that the DEP can be used to separate strain B from the other three strains.

The severity of leaf necrosis in plants infected with certain of the strains might be associated with virus concentration. The lower relative concentration of strain B could explain the absence of leaf necrosis in plants infected with this strain. The high relative concentration of strain A could explain the severity of the leaf necrosis on plants infected with this strain. It is also possible that production of necrosis is a property of the virus-host interaction not dependent on concentration. In any event, it may be possible to use the degree of leaf necrosis on beefbuilder sorghum to distinguish strain B from other strains.

Table 5 shows the results of a comparative study of three methods of purification for SCMV. The data show that the chloroform-water procedure gave the highest amount of infectious virus. With the acid clarification and the chloroform buffer procedures, a major loss of infectivity occurred. The loss of infectivity can probably be attributed to virus aggregation. Shepherd and Pound (67), van Regenmortel et al. (81) and Delgado-Sanchez and Grogan (26) found aggregation a major problem during extraction and purification of viruses in the potato virus Y (PVY) group. Van Regenmortel (80), reported that considerable packing occurs with the rod-shaped plant viruses during centrifugation.

According to Delgado-Sanchez and Grogan (26), their procedure reduced the amount of aggregation of PVY considerably. This may explain the higher amount of infectivity obtained with SCMV using this method.

It is interesting to note that with the chloroform-buffer procedure infectivity was lower in the initial step (homogenization) than with the other two procedures. With this procedure, the tissue was homogenized in 0.5M sodium citrate. According to Scott (64), some viruses will break up into subunits if the ionic strength of the buffer used approaches or exceeds 0.2M. This may explain the lower infectivity in the initial step with this procedure.

It is also interesting to note that chloroform clarification appeared to reduce infectivity with the chloroform-buffer procedure, but not with the chloroform-water procedure. This difference may be due to the presence of ascorbic acid and SDDC in the latter procedure. Apparently these materials protect the virus particles in some manner from chloroform inactivation.

Typical ultraviolet absorption spectrums were not obtained with material resulting from either the acid or the chloroform-buffer procedure. However, typical UV absorption spectrums were obtained with material resulting from the chloroform-water procedure. This indicates that virus obtained using the latter procedure had less host contaminating material than with the other two procedures.

Differences were observed between the various strains and their reaction to the different purification procedures. Using scanning patterns from the density-gradient fractionator as a measure of relative virus yields, Strains A, D, and H gave relatively high yields

with each purification procedure. Numerous attempts at purification of strain B and the Johnson grass mosaic with the acid clarification method failed. The failure to purify strain B with this method could also be due to the low concentration of strain B in the host plant. Since greater losses are incurred with the acid procedure and the chloroform-buffer procedure, virus present in low concentration initially might all be lost during purification. This could also account for the failure to purify the Johnson grass mosaic with this procedure. Another possibility is that strain B and the Johnson grass mosaic may have properties different from those of the other three strains. More aggregation may have occurred using this method of purification.

II. Serology of Strains of SCMV

Data in Table 6 show the results of microprecipitin tests of strains A, B, D, and H of SCMV. The Johnson grass strain was included to determine its relationship to SCMV. The homologous titers of the antisera to strains A, B, D, and H were 1/128, 1/256, 1/125, and 1/256, respectively. The heterologous precipitin end point showed slight variations, but never varied more than one dilution from the homologous reaction, except the heterologous reaction involving strain H antigen and strain A antiserum. The antisera reacted with healthy sorghum protein up to 1/8, but the precipitate was easily distinguished from that produced by the virus. These data indicate that strains A, B, D and H are closely related serologically and cannot be differentiated in microprecipitin tests.

The Johnson grass strain reacted with strain H antiserum up to 1/64 dilution. Thus, the Johnson grass mosaic present in Louisiana

is serologically related to SCMV, but not as closely as the strains are to each other. These results are similar to those reported by other workers (11, 83).

Figures 2-10 show the results of the agar gel diffusion tests. The figures show the reaction of each antiserum with the respective heterologous and homologous antigens. When antisera of strains A, B, D, and H were tested against the degraded virus (antigen) of the four strains, all antisera reacted against antigens of strain A, D and H. The zones produced by these antigens banded together indicating complete serological identity. Antigen of strain B did not react to any antisera including its own, although its antiserum reacted against A, D, and H antigens. The low concentration of strain B in the host plant as shown in DEP studies and the low concentration of purified virus obtained could account for the failure to obtain a reaction with this strain. Again, this may indicate a difference between this strain and the other strains of SCMV. Antigen of the Johnson grass mosaic gave a very weak reaction with antisera to the strains of SCMV and spurring occurred (Figure 10). This indicates that the Johnson grass mosaic has certain antigenic sites not common to the other strains of SCMV. Thus, it may be concluded that Johnson grass mosaic in Louisiana is a serologically distinct strain of SCMV.

III. Soil Transmission of SCMV

Data in Table 7 show the results of initial tests designed to demonstrate soil transmission of SCMV. In the first five experiments, transmission ranged from .7 to 5.4%. In the first five experiments,

two plants became infected in the controls. It seems likely that this was due to contamination, since no attempt was made to avoid water splashing into the pans containing the control plants. In the last four experiments, care was taken to avoid water splashing into the controls. Here, transmission to the test plants ranged from .8 to 5%. There was no transmission to the control plants. These data indicate that transmission occurred from infected plants to noninoculated plants through the soil. In these experiments no attempt was made to keep the roots of infected plants separate from those of the noninoculated plants. Thus, root contact could have accounted for the transmission in these experiments. According to Harrison (36), soil-borne viruses are those "with an underground method of natural spread which does not depend simply on contact between tissues of infected and healthy plants." Data in Tables 8 and 9 show that root contact is not necessary in order to obtain transmission. In the first experiments (Method A), transmission to plants grown in soil adjacent to inoculated plants was 4.5 and 8.8%. Transmission of plants grown in porcelain pans into which water was allowed to wash was 6.2%. In pans which were watered separately, there was no transmission. Data in Table 9 also demonstrates that root contact is not necessary in order for transmission to occur. Transmission was about 5% in all replications.

Since SCMV is an aphid transmissible virus, experiments were made under screen cages to rule out any possibility of aphid transmission. Data in Table 10 show the results of this experiment. With Method A, transmission ranged from 1.6 to 10.1%. With Method B, transmission ranged from .9 to 2.5%.

The mechanism of transmission of SCMV through the soil is unknown. Attempts made to correlate the presence of a biological vector with transmission were all negative. A severe root and lower stem necrosis was observed on most of the sorghum plants (Plate 2). There was a general correlation between the severity of the root necrosis and transmission. What role the root necrosis plays in transmission is not known. One possible explanation is that the virus is simply released from infected plants into the soil. This virus could then enter noninoculated plants through the necrotic areas in the roots. Yarwood (87) reported virus release from roots of plants infected with tobacco necrosis virus (TNV) and tobacco mosaic virus (TMV). Grogan and Campbell (35) do not agree with the hypothesis that soil transmission occurs through wounds produced by roots growing through soil or sand. These authors state that it is practically impossible to maintain freedom from a fungus, such as Olpidium, under ordinary greenhouse conditions. For this reason, they are of the opinion that accidental contamination could account for reported transmission of viruses in the absence of a biological vector.

SUMMARY

1. Studies were made to determine if strains A, B, D and H of SCMV could be differentiated on the basis of physical, chemical and biological properties.
2. Studies of the physical properties of the four strains showed that thermal inactivation points (TIP) are of no value in strain differentiation. All strains were still active at $55^{\circ}C$, but not at $57^{\circ}C$.
3. Dilution end point (DEP) studies showed a difference in certain of the strains in their tolerance to dilution. Strain A and H were still infectious at 10^{-4}, strain D at 10^{-3}, strain B at 10^{-2}.
4. A severe leaf necrosis developed on plants infected with certain of the strains. Necrosis occurred on plants infected with strains A, D, and H, but not on plants infected with strain B. There was a correlation between the presence of the leaf necrosis and virus concentration.
5. Three methods of purification were compared to determine the one best suited for use with SCMV. A modification of the method of Delgado- Sanchez and Grogan for potato virus Y (PVY) yielded the highest amount of infectious virus. Virus purified by this method had less host contaminating material than with other methods tested.
6. Microprecipitin tests could not be used to differentiate any of the strains. In these tests, all strains appeared to be closely related.

7. Agar diffusion tests showed that strains A, D, and H are closely related. Antigen of strain B did not react with antisera to any of the strains including its own.
8. Microprecipitin and agar diffusion tests showed that the Johnson grass mosaic in Louisiana is serologically related to SCMV, but not as closely as are the strains to each other. Spurring in agar diffusion tests showed that it is a distinct strain of SCMV.
9. Studies showed that SCMV can be transmitted from infected plants to noninoculated plants through the soil. Transmission occurred in the absence of root contact. The involvement of a biological vector in soil transmission remains to be demonstrated.

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EXAMINATION AND THESIS REPORT

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Major Field: Plant Pathology

Title of Thesis: Chemical, Physical and Biological Properties of Four Strains of Sugarcane Mosaic Virus

Approved:

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